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TITLE: In Vivo Measurement of Drug Efficacy in Breast Cancer

PRINCIPAL INVESTIGATOR: Dr. Randy J. Giedt

CONTRACTING ORGANIZATION: MASSACHUSETTS GENERAL HOSPITAL
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PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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AND ADDRESS(ES)

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The focus of this project is to understand how how nano-encapsulated formulations of common chemotherapies work in vivo by developing and utilizing intravital methods for studying drug and nanoparticle function in mouse breast cancer models. We hypothesize that, firstly, we can develop longitudinal breast cancer specific methods of imaging common chemotherapies and their nanoparticle equivalents. Secondly, we hypothesize that encapsulated drugs will be more effective in terms of specific cell responses as they achieve longer exposure times than unencapsulated drugs. Overall, this work will result in the creation of a breast cancer centered platform for drug development and analysis. At the clinical level, this study will result in pertinent data regarding several agents currently in clinical trials. At the basic science level, we will work to understand the heterogeneity of cell responses to drug treatments. Thus, we believe this project has potential impact in both the near and long term for breast cancer treatment.

15. SUBJECT TERMS

Breast Cancer, Intravital Imaging, Nanoparticles, Pharmacokinetics/ Pharmacodynamics, Chemotherapy, Drug Distribution

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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Table of Contents

1. INTRODUCTION

The focus of this project is to understand how how nano-encapsulated formulations of common chemotherapies work in vivo by developing and utilizing intravital methods for studying drug and nanoparticle function in mouse breast cancer models. We hypothesize that, firstly, we can develop longitudinal breast cancer specific methods of imaging common chemotherapies and their nanoparticle equivalents. Secondly, we hypothesize that encapsulated drugs will be more effective in terms of specific cell responses as they achieve longer exposure times than unencapsulated drugs. Overall, this work will result in the creation of a breast cancer centered platform for drug development and analysis. At the clinical level, this study will result in pertinent data regarding several agents currently in clinical trials. At the basic science level, we will work to understand the heterogeneity of cell responses to drug treatments. Thus, we believe this project has potential impact in both the near and long term for breast cancer treatment. During Year 2, this project has focused on developing drug and NP drug delivery methods for testing in animal models, for follow on imaging and analysis in year 3 of this project.

2. KEYWORDS

Breast Cancer, Intravital Imaging, Nanoparticles, Pharmacokinetics/ Pharmacodynamics, Chemotherapy, Drug Distribution

3. ACCOMPLISHMENTS

What were the major goals of the project?

The major goals of this project for year 2 have focused on the setup of fluorescent drugs, Nanoparticles (NPs), and animal testing methods for follow on analysis in Year 3 of this grant.

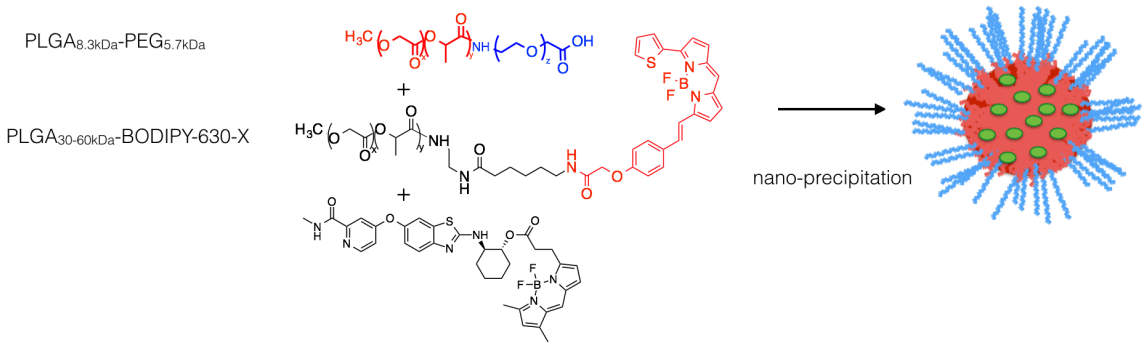
Table 1: Specific Tasks for Grant Aim 1

Specific Aim 2: Investigate Targeted Nanoformulations for drug delivery	Months	GSU	% Complete
Subtask 2a: In vitro validation and comparison of nano encapsulated and unencapsulated versions of drugs across breast cancers.	12-15	Dr. Giedt	100%
Subtask 2b: Evaluate Drugs and NP formulations in Mice	15-22	Dr. Giedt	100%
Subtask 2c: Analyze and model data utilizing previously created algorithms and methods.	22-24	Dr. Giedt	100%

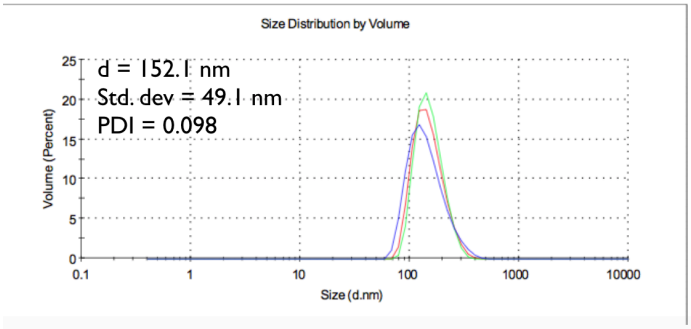
What was accomplished under these goals?

1. **In vitro validation and comparison of nano encapsulated and unecapsulated drugs across breast cancers (100% Complete):**

The goal of this sub-aim was to fully develop and verify methods for encapsulating a variety of drugs in nanoparticles to determine which of these would be most appropriate/ promising for follow on studies. In line with reviewer comments for this grant concerning “a lack of novel agents” we have tested both classic agents already in the clinic, as well as agents found in emerging trials to address reviewer comments. A typical nanoparticle encapsulation formulation is illustrated below.



Ratio PLGA_PEG : PLGA_BODIPY630 : BLZ945_BODIPY ester = 10:2:1



Concentration of BLZ945_BODIPY ester in NPs:
(4.8 mg/mL, 7.1 mM (effectively 2.8 mg/mL of BLZ945)

Dose per 150 µL injection would be *ca* 17 mg/kg

Figure 1: Typical Nanoparticle encapsulation scheme. Drugs such as BLZ-945 were encapsulated in PLGA-PEG NP formulations following chemical modification in various forms (see below). Characterization of NPs was completed analyzing the size distribution of NPs (see bottom left) and the concentration of NPs in a given volume as well as the particle distribution size. Finally, dosing amounts for an average sized mouse were calculated.

A complete list of drugs tested, describing success or failure of these agents in PLGA-PEG nano formulations is found in Table 2. Our selection of agents was based on both availability of drugs, their likelihood of success in nanoparticle formulations, and their clinical utility in breast cancer.

As a model agent to derive optimal chemical modifications for these agents, we utilized BLZ-945 (due to optimal chemical properties for modification) and tested an assortment of chemical modifications. Results are shown in Table 3, comparing chemical modifications to effects of the drug response, encapsulation efficiency and amount of drug in a 200 uL NP suspension. We anticipate extrapolating these results to attempt to derive generalizable qualities in drugs which we could utilize to improve nanoparticle efficiency in various drug types. A final decision on drug types for full study will be made in year 3 of this grant.

2. In vitro testing

To test drugs in an in vitro environment, various cell line assays have been utilized. For BLZ-945, we tested cells in a lymphoma model overexpressing the protein target, CSF-1R, with various forms of the drug via a Presto-Blue assay (Figure 2). For other drugs, such as Afatinib

Drug	Successful/ Mouse Dosing
Cisplatin	Yes, Dosing at therapeutic levels
BLZ-945	Yes, Dosing at 10 mg/kg Mouse
Taxol	Yes, Dosing at therapeutic Levels
ABT-199	Yes, Dosing below therapeutic levels
ABT-263	Yes, Dosing at therapeutic levels
Afatinib	Yes, Dosing at therapeutic levels
GW2580	Yes, Low therapeutic dosing levels
OSI-930	Yes, Low therapeutic dosing levels
Linifanib	Yes, Low therapeutic dosing levels
CEP-32496	Yes, Low therapeutic dosing levels

Table 2: Drugs tested in nanoparticle formulations. Cisplatin and Taxol are agents typically utilized in the clinic. BLZ-945, GW2580, OSI-930, Linifanib, and CEP-32496 are tumor microenvironment targeting agent found in clinical trials. Similarly, ABT-199 and ABT-263 are agents found in clinical trials, targeted to the apoptosis pathway. Afatinib is an EGFR targeting agent.

were tested with engineered high EGFR cell lines, for example. Each drug conjugate completed has been fully tested.

Additionally, in conjunction with chemists in the lab I have continued to develop fluorescent reporters of these specific drugs as seen in year 1. One such fluorescent conjugate in testing is seen below. In total, we have successfully created fluorescent reporter cell lines for BLZ-945, cisplatin, taxol, and afatanib from the table above. Other additional conjugates are also in construction for testing in nanoparticle formulations, but are not needed for the successful completion of this grant.

Subtask 2b. Test Drugs and NP formulations in Mice.
In this goal I have worked to develop testing procedures to verify NP drug conjugates in mice. We have again utilized the work conducted in BLZ-945 drug as a model system. Animal testing was conducted in Balb/C mice with the mouse breast cancer model cell line of 4T1 cell lines. The goal of these experiment was to analyze, 1. If nanoparticle encapsulation creates an improved therapeutic efficiency as compared to various concentrations of the unencapsulated drug, and 2. Determine if NPs by themselves lead to drug toxicity or if alternate forms of drugs may be more efficacious in NPs. Therefore, in our first experiment, concentrations of either 2 mg/ kg BLZ-945, 10 mg/kg BLZ-945 or encapsulated drug were tested in BALB/C mice expressing tumors in mammary fat pads, showing limited additional efficacy as seen (Fig. 4).

Following these experiments, we tested conjugates of BLZ-945 as described in table 3 for their efficacy in NP form. As seen in Figure 5, these form a promising additional treatment strategy,

Compound	cLogP (Chemdraw)	IC50 (nM) "Naked"	IC50 (nM) PLGA_PEG encapsulated	Concentration (mM) of drug in 200 μL NP suspension
BLZ945	3.68	164	455	2.1
BLZ945-acetate	4.58	283	178 (234)	2.4
BLZ945-propionate	5.10	138	187	5.4
BLZ945-heptanoate	7.22	247	ND	ND
BLZ945-decanoate	8.81	446	593	3.8
BLZ945-C16 ester	12.0	2240	4970** (estimated)	ND

Table 3: Drug conjugates tested in NP formulations for the model system of BLZ-945. Chemical modifications for BLZ-945 were made via addition of various chemical additions and then the encapsulation efficiency was tested to determine optimal chemical properties for the system, as determined by encapsulation efficiency and maximum dosing levels.

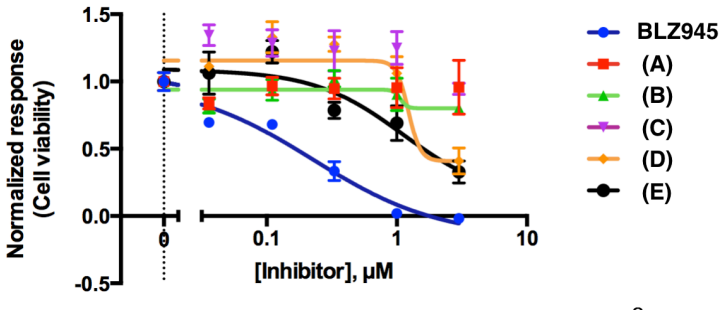


Figure 2: Cell growth assay for BLZ-945, with conjugates from Table 3 (A-E) including acetate, propionate, heptanoate, decanoate, and C16 ester.

likely due to their additional efficacy in terms of increasing NP encapsulation efficiency, at least in the Balb/C model presented here.

Animal experiments are currently ongoing in other drug and NP models as well. In Year 3 of this grant, we will make a final decision on which specific drugs to test out of those tested above.

Subtask 2c. Develop and utilize mathematical models for drugs.
Imaging and analysis methods for this grant were fully completed in Year 1 of the grant. These methods have been employed in analyzing both drug efficacy of nanoparticles as well as in vitro data as seen in 2a. and 2b.

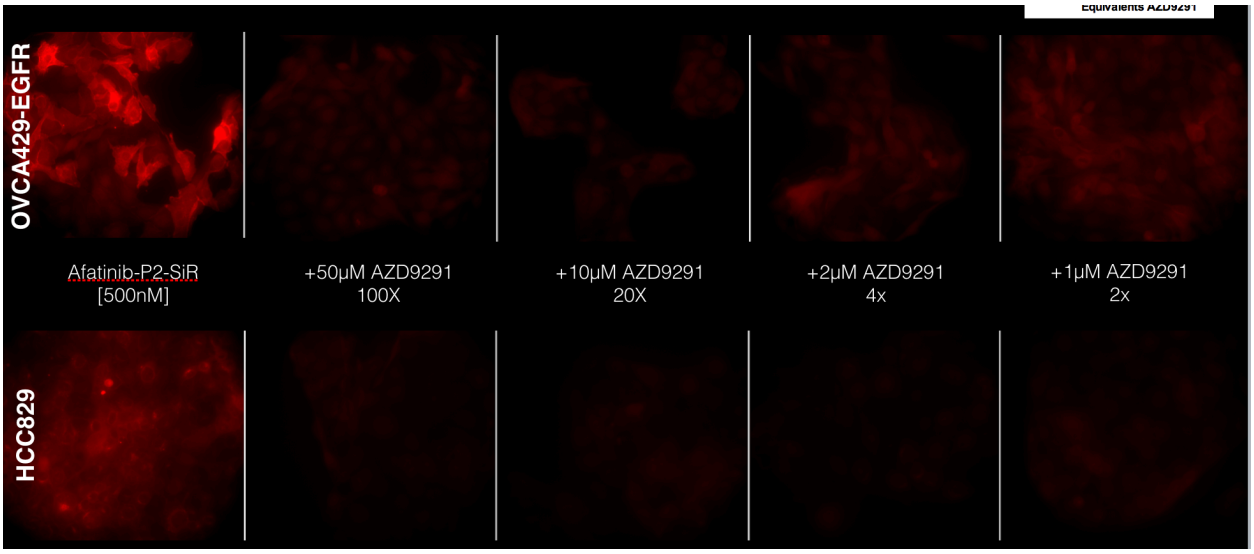


Figure 3: Fluorescent conjugate testing for afatanib. A fluorescent version of afatanib was tested against OVCA-429 EGFR, an engineered EGFR fluorescent cell line, and HCC829, a naturally occurring EGFR over expressing cell line. Drugs were tested against AZD9291, an EGFR targeting drug, to ensure on target effects of the fluorescent conjugates in a competition experience (in 100X, 20X, 4X and 2X excesses to the fluorescent drug).

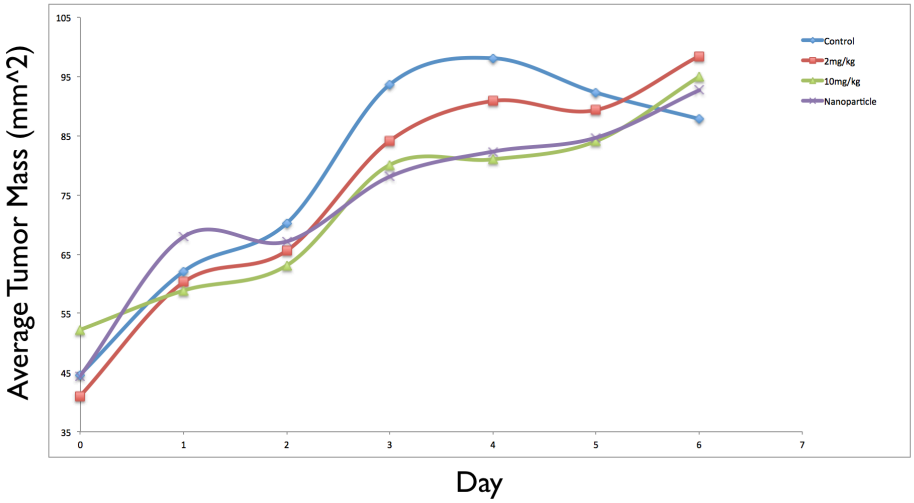


Figure 4. Mouse tumor growth experiments. Experiments were conducted in a BALB/C Mouse model with either unencapsulated drug in two differing concentrations, or encapsulated drug, all utilizing the BLZ-945 tumor growth model with normal formulation of BLZ-945, showing limited efficacy of the nanoparticle model.

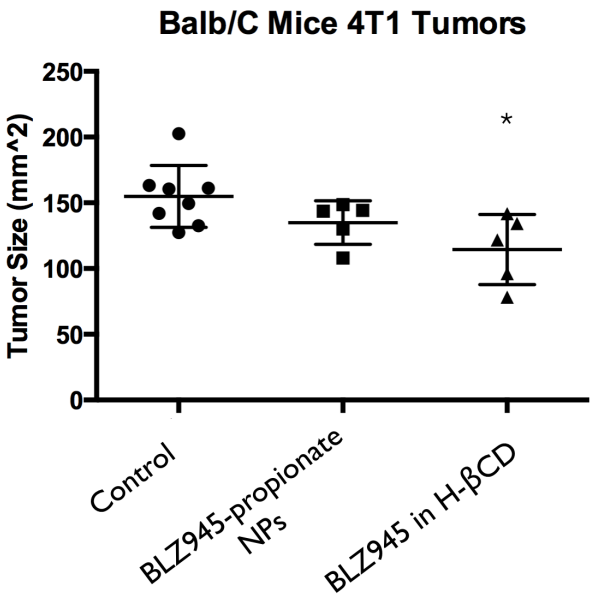


Figure 5. Mouse tumor growth experiments with altered drug and delivery mechanisms. Experiments were conducted in a BALB/C Mouse model with either BLZ-945-proprionate, BLZ945 in H-BCD or control drugs, all utilizing the BLZ-945 tumor growth model with normal formulation of BLZ-945, showing limited efficacy of the nanoparticle model.

What opportunities for training and professional development has the project provided?

Table 4: SOW Goals for training and professional development.

Major Task 1: Training and educational development in breast cancer research	Months	GSU	% Complete
Subtask 1: Attend bi-weekly scientific talks at MGH focusing on cancer biology.	1-36	Dr. Giedt	100
Subtask 2: Attend monthly scientific talks focusing on apoptosis and cancer (Harvard Medical School - Longwood)	1-36	Dr. Giedt	100
Subtask 3: Attend tri-monthly scientific “T-32 Trainee” scientific talks focusing on cancer biology.	1-36	Dr. Giedt	100
Subtask 4: Attend and present at yearly cancer/ breast cancer meetings (e.g. AACR, ASCO, others).	1-36	Dr. Giedt	100

Major Task 1: Training and educational development in breast cancer research	Months	GSU	% Complete
Subtask 5: Attend breast cancer conferences at the DF Harvard Cancer center (and others if available, e.g. at MSKCC) and present data/posters	1-36	Dr. Giedt	100
Subtask 6: Present ongoing project results for feedback in weekly lab meetings.	1-36	Dr. Giedt	100
Subtask 7: Weekly meetings with mentor and co-mentor	1-36	Dr. Giedt	100
Subtask 8: Attendance at 3 Responsible Conduct of Research Meetings per year. Attend all MGH OCRD (Office of Career Development) meetings on faculty development	1-36	Dr. Giedt	100
Milestone Achieved: Presentation of project data at a national meeting	12,24,36		

This project has provided numerous opportunities for professional development, consistent with the training plan established in the accepted grant proposal. Specific numbers of lecture sessions attended in the MGH/ Harvard community is presented in Table 4 summarizing relevant scientific discussions. Briefly, these lectures focused on Breast Cancer to the extent possible, cancer in general, and new technologies and animal manipulation methods which are directly applicable to project goals.

Table 4: Number of Lectures attended for required educational opportunities.

Lecture Series	Location	Number of Lectures Attended
Center for Systems Biology Scientific Talks	Mass General, Simches, 3rd Floor	9
Mass General Hospital Cancer Center Grand Rounds	Mass General, Simches, 3rd Floor	~ 20
NIH T32 Post-doctoral Fellow Lectures	Mass General, Haber Auditorium, Blake 1	4
Harvard Department of Systems Biology Apoptosis Meeting	Harvard Medical School, 563 Warren Alpert	11
Mass General Hospital Responsible Conduct of Research Lectures	Mass General, Variable	4
Mass General Hospital Office of Career Development Lectures	Mass General, Variable	3
MGH Systems Biology Lab Meetings	Mass General, Simches, 5th Floor	~ 40

Lecture Series	Location	Number of Lectures Attended
JAX Onsite Animal Training	Mass General, Simches 3rd Floor	2

In addition to regular lectures, this grant also enabled travel to one American Association for Cancer Research Meeting (main meeting held in New Orleans this year), where the PI was awarded a scholar in training award from Bristol Meyers Squibb for an outstanding abstract.

How were the results disseminated to communities of interest?
Thus far preliminary results from this project have been discussed in weekly lab group lab meetings. Primary results from this project will be published with the completion of imaging experiments.

What do you plan to do during the next reporting period to accomplish the goals?
In this reporting period we have focused on developing NP formulations for a number of drugs, and testing them against breast cancer models to determine the most promising of these for follow-on development. During the next reporting period, we plan to focus on imaging drugs and conducting full mathematical modeling for the best breast cancer focused drug of those described above (likely BLZ-945 based on our preliminary results presented above). We will then publish this work to complete the project.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?
Nothing to report.

What was the impact on other disciplines?
Nothing to report.

What was the impact on technology transfer?
Nothing to report.

What was the impact on society beyond science and technology?
Nothing to report.

5. CHANGES/ PROBLEMS

Changes in approach and reasons for change?
We anticipate the approach will remain the same as described in the original award.

Actual or anticipated problems or delays and actions or plans to resolve them?
No delays or problems were encountered during this reporting period.

Changes that had a significant impact on expenditures?
There were no changes to expenditures.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/ or select agents?
There are no changes to report in the use of animals, biohazards or select agents.

6. PRODUCTS

Journal publications
Due to this training award, PI was published on the following papers during this funding period:
Published Papers:

1. **Giedt RJ**, Fumene-Fergulio P, Pathania D, Yang KS, Kilcoyne A, Vinegoni C, Mitchison TJ, Weissleder R. Computational imaging reveals mitochondrial morphology as a biomarker of cancer phenotype and drug response. *Sci Rep.* 2016 Sep 9;6:32985. doi: 10.1038/srep3298.

2. Meimetis LG*, **Giedt RJ***, Mikula H, Carlson JC, Kohler RH, Pirovich DB, Weissleder R. Fluorescent vinblastine probes for live cell imaging. *Chem Commun* (Camb). 2016 Aug 2;52(64): 9953-6. doi: 10.1039/c6cc04129a.

3. Dubach JM, Kim E, Yang K, Cuccarese M, **Giedt RJ**, Vinegoni C, Weissleder R. Quantitating drug-target engagement in single cells in vivo. *Nature Chem Bio. In Press.*

4. Leon-Swisher C, Vinegoni C, Fumene-Feruglio P., **Giedt RJ**, Rousso DL, Weissleder R. Real-time high dynamic range laser scanning microscopy. *Nature Comm*. 2016 Apr 1;7:11077. doi: 10.1038/ncomms11077.

Books or other non-periodical, one-time publications
Nothing to report.

Other publications, conference papers, and presentations
Conference Presentation:
AACR - Mitochondrial Morphology as a Biomarker in Cancer. New Orleans, April, 2016.
Awarded AACR Bristol-Meyers-Squibb Scholar in Training Award for this presentation.

Websites or other Internet site(s)
Nothing to report.

Technologies or techniques
Nothing to report.

Inventions, patent applications and/or licenses
Nothing to report.

Other products
Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name	Randy J Giedt
Project Role	PI/ Research Fellow
Researcher Identifier	ORCID 0000-0001-8327-6069
Nearest person month worked	12
Contribution	Dr. Giedt is the PI of this post-doctoral Fellowship and as such has conducted all research on this grant as well as attending required trainings as described in the original application.
Funding Support	DOD BCRP Post-doctoral Fellowship

Has there been a change in the active other support of the PD/PI(s) or senior/ key personnel since the last reporting period?

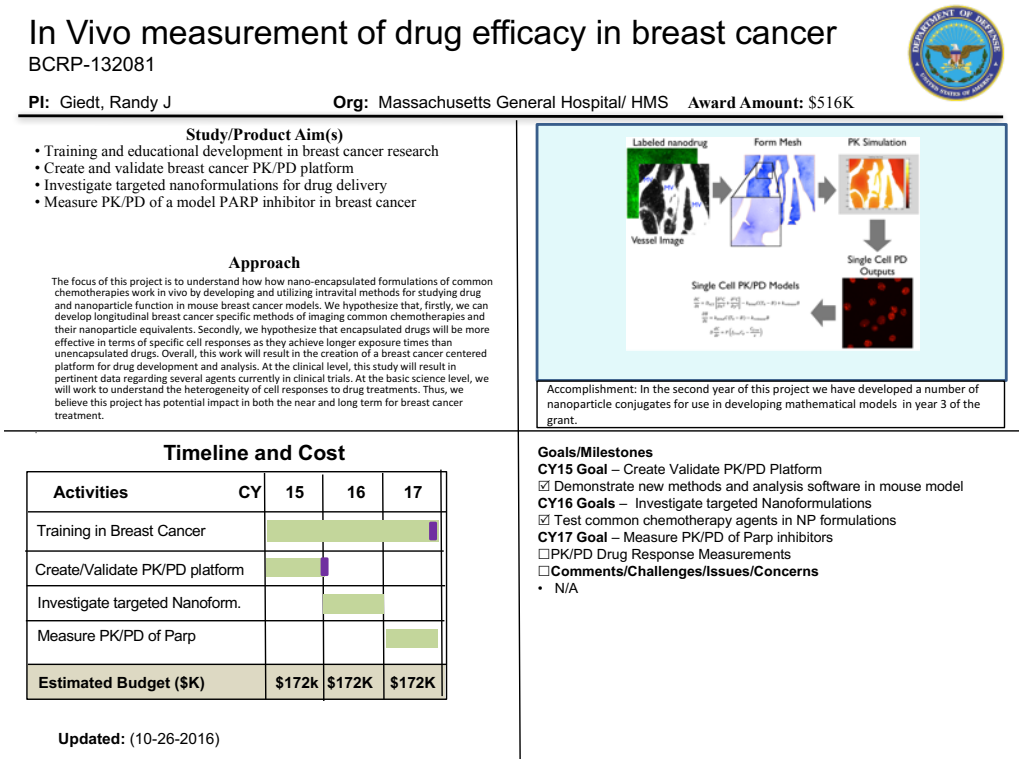
No changes have occurred in the PIs funding for this project.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

Quad Chart:



In Vivo measurement of drug efficacy in breast cancer

BCRP-132081

PI: Giedt, Randy J **Org:** Massachusetts General Hospital/ HMS **Award Amount:** $516K

Study/Product Aim(s)

- Training and educational development in breast cancer research
- Create and validate breast cancer PK/PD platform
- Investigate targeted nanoformulations for drug delivery
- Measure PK/PD of a model PARP inhibitor in breast cancer

Approach

The focus of this project is to understand how how nano-encapsulated formulations of common chemotherapies work in vivo by developing and utilizing intravital methods for studying drug and nanoparticle function in mouse breast cancer models. We hypothesize that, firstly, we can develop longitudinal breast cancer specific methods of imaging common chemotherapies and their nanoparticle equivalents. Secondly, we hypothesize that encapsulated drugs will be more effective in terms of specific cell responses as they achieve longer exposure times than unencapsulated drugs. Overall, this work will result in the creation of a breast cancer centered platform for drug development and analysis. At the clinical level, this study will result in pertinent data regarding several agents currently in clinical trials. At the basic science level, we will work to understand the heterogeneity of cell responses to drug treatments. Thus, we believe this project has potential impact in both the near and long term for breast cancer treatment.

Accomplishment: In the second year of this project we have developed a number of nanoparticle conjugates for use in developing mathematical models in year 3 of the grant.

Timeline and Cost

Activities / CY	15	16	17
Training in Breast Cancer			
Create/Validate PK/PD platform			
Investigate targeted Nanoform.			
Measure PK/PD of Parp			
Estimated Budget ($K)	**$172k**	**$172K**	**$172K**

Updated: (10-26-2016)

Goals/Milestones

CY15 Goal – Create Validate PK/PD Platform
☑ Demonstrate new methods and analysis software in mouse model
CY16 Goals – Investigate targeted Nanoformulations
☑ Test common chemotherapy agents in NP formulations
CY17 Goal – Measure PK/PD of Parp inhibitors
☐PK/PD Drug Response Measurements
☐**Comments/Challenges/Issues/Concerns**

- N/A

9. APPENDICES

1. PI Updated CV

Randy James Giedt, PhD
3610 Mystic Valley Parkway, Apt. 304N
Medford MA, 02155

EDUCATION

The Ohio State University
Columbus, OH
Doctor of Philosophy, Biomedical Engineering
June 2012

The Ohio State University
Columbus, OH
Master of Science, Biomedical Engineering
June 2009

South Dakota State University
Brookings, SD
Bachelor of Science, Mechanical Engineering, *Cum Laude*
May 2007
- *Minor degree*, Biology

RESEARCH EXPERIENCE

Massachusetts General Hospital, Harvard Medical School
Boston, MA
Post-doctoral Fellow/ Research Fellow (Center for Systems Biology) *August 2012 – Current*
Postdoctoral mentor: Ralph Weissleder, M.D., Ph.D.

The Ohio State University, Biomedical Engineering Department
Davis Heart and Lung Research Institute
Columbus, OH
Graduate Research Associate (Vascular Mechanotransduction Laboratory)
2007 – 2012
Thesis advisor: B. Rita Alevriadou, Ph.D.

Hub City Manufacturing, Engineering Department
Aberdeen, SD
Engineering Intern
Summers 2005, 2006

TEACHING EXPERIENCE

The Ohio State University, Department of Biomedical Engineering
Columbus, OH
Graduate Teaching Assistant
2009-2010

- Assisted with courses including Numerical Simulations in BME, Biomaterials, Biotransport and Introduction to Matlab.

The Ohio State University, Department of Mechanical Engineering
Columbus, OH
Graduate Teaching Assistant
Fall 2007

- Instructed engineering Measurements Lab (Signal processing, pressure measurements, and fluid flow measurements).

Fellowship and Grant Support

1. 2014-2017 Congressionally Directed Medical Research Programs (CDMRP): Department of Defense Breast Cancer Research Program Post-Doctoral Fellowship ($300,000 Direct + Indirect). Conceived the research plan, and wrote the grant application (under the guidance of Dr. Ralph Weissleder).

2. 2011-2012 American Heart Association Pre-doctoral Fellowship ($23,000 Direct). Conceived the research plan, and wrote the grant application (under the guidance of Dr. B. Rita Alevriadou).

3. *Submitted* Oct. 12, 2016. NIH NCI K22 Transition Career Development Award.

AWARDS AND HONORS

1. 2016 AACR-Bristol Myers Squibb Oncology Scholar-in-Training Award. American Association of Cancer Research.

2. 2011 Best Overall Presentation, The Ohio State University Biomedical Engineering Department Research Conference.

3. 2010 1st Place: The Ohio State University Edward F. Hayes Graduate Research Forum, Science and Technology Poster Division.

4. 2009 Travel Award from the Biomedical Engineering Society (BMES).

5. 2009 Best Poster Presentation under the "Molecular, Cellular, & Tissue Engineering" track, The Ohio State University Biomedical Engineering Department Research Conference.

PEER REVIEWED PUBLICATIONS

1. **Giedt RJ**, Fumene-Fergulio P, Pathania D, Yang KS, Kilcoyne A, Vinegoni C, Mitchison TJ, Weissleder R. Computational imaging reveals mitochondrial morphology as a biomarker of cancer phenotype and drug response. *Sci Rep.* 2016 Sep 9;6:32985. doi: 10.1038/srep3298.

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5. Yang KS, Kohler RH, Landon M, **Giedt RJ**, Weissleder R. Single-cell Pharmacodynamic imaging of Parp inhibitor efficacy. *Sci Rep.* 2015; 5:10129.

6. Turestsky A, Kyungheon L, Song J, Castro C, **Giedt RJ**, Kovach A, Hochber E, Lee H, Weissleder R. On chip analysis of CNS Lymphoma in Cerebrospinal Fluid. *Theranostics.* 2015; 5(8): 796-804.

7. Giedt RJ*, Sprachman MM*, Yang KS, Weissleder R. Imaging Cellular Distribution of Bcl Inhibitors using Small Molecule Drug Conjugates. *Bioconjugate Chem*. 2014 Nov 19; 25(11): 2081-5.

8. Alieva M, Ritsma L, **Giedt RJ**, Weissleder R, van Rheenen J. Imaging windows for long-term intravital imaging: General overview and technical insights. *Intravital.* 11 Aug. 2014.

9. Meimetis LG, Carlson JC, **Giedt RJ**, Kohler RH, Weissleder R. Ultrafluororenic coumarin-tetrazine probes for real-time biological imaging. *Angew Chem Int Ed Engl*. 2014 Jul 14;53(29): 7531-4.

10. Kim E, Yang KS, **Giedt RJ**, Weissleder R. Red Si-rhodamine drug conjugates enable imaging in GFP cells. *ChemComm*. 2014 May 4:50(34):4504-7.

11. Coffey SE*, **Giedt RJ***, Weissleder R. Automated analysis of clonal cancer cells by intravital imaging. *Intravital*. 2013 Jul;2(3). *Denotes 1st co-authorship.

12. Giedt RJ, Koch PD, Weissleder R. Single Cell Analysis of Drug Distribution by Intravital Imaging. *PLoS One*. 2013 Apr 10;8(4).

13. Giedt RJ, Pfeiffer DR, Matzavinos A, Kao CY, Alevriadou BR. Mitochondrial dynamics and motility inside living vascular endothelial cells: role of bioenergetics. *Ann Biomed Eng*. 2012 Sep;40(9):1903-16.

14. Giedt RJ, Yang C, Zweier JL, Matzavinos A, Alevriadou BR. Increased mitochondrial fission in endothelial cells following simulated ischemia/reperfusion: Role of nitric oxide and mitochondrial reactive oxygen species. *Free Radical Medicine and Biology.* 2012 Jan 15; 52(2): 348-56.

15. Han Z, Varadharaj S, **Giedt RJ**, Zweier JL, Szeto HH, Alevriadou BR. Mitochondria-derived reactive oxygen species mediate heme oxygenase-1 expression in sheared endothelial cells. *J Pharmacol Exp Ther*. 2009; 329(1):94-101.

* Denotes 1st co-authorship.

BOOK CHAPTERS

1. Giedt RJ, Yang KS, Weissleder R. *Imaging Drug Distribution and Effects at the Single Cell Level In Vivo*. Advances in Intravital Microscopy: From Basic to Clinical Research. Weigert R. Springer Publishing Company, New York City New York, 2014, pp. 263 – 280.

2. B.R. Alevriadou, C.I. Jones 3rd, **R.J. Giedt**. *Nitric oxide and endothelial mitochondrial function: Implications for ischemia/reperfusion*. Hemodynamics & Mechanobiology of Endothelium, Hsiai

TK, Blackman B, and Jo H, World Scientific Publishing Co., Singapore & USA, September 2010, pp. 153-177.

MANUSCRIPT REVIEWS

Ad hoc peer reviewer for manuscripts submitted to: *Molecular Pharmaceutics, Scientific Reports.*

SELECTED PRESENTATIONS

- **Giedt RJ**, Weissleder R. Mitochondrial morphology as a biomarker of cancer phenotype and drug response. Biomedical Engineering Society Annual Meeting, Minneapolis, MN, Oct. 13, 2016.
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- **Giedt RJ**. Mitochondrial morphology as a biomarker of cancer phenotype and drug response. Graduate student seminar, Harvard University Department of Systems Biology. February 23, 2016.

- **Giedt RJ**, Feruglio PF, Pathania D, Mitchison TJ, Weissleder R. Single Cell Analysis of Mitochondrial Morphology in Cancer. Harvard Systems Biology Departmental Retreat, May 2015 (Seabasco Bay, ME).

- **Giedt RJ**, Weissleder R. Mitochondrial Morphology: Predictor for Cancer Therapy. Harvard Systems Biology Apoptosis Meeting, March 2014 (Boston, MA).

- **Giedt RJ,** Koch PD, Weissleder R. Single Cell Analysis of Drug Distribution by Intravital Imaging. Scientific Advisory Committee Meeting, Massachusetts General Hospital, May 2013 (Boston, MA).

- **Giedt RJ,** Weissleder R. Intravital Imaging Effects of Bcl2 Inhibitors. Harvard Systems Biology Apoptosis Meeting, November 2012.

- **Giedt RJ**, Yang C, Zweier JL, Matzavinos A, Alevriadou BR. Mitochondrial Changes in Endothelial Cells Due to Mechanochemical Stimuli. Presented at the Biomedical Engineering Society (BMES) Annual Meeting, October 2011 (Hartford, CT).

- **Giedt RJ**, Pfeiffer DR, Matzavinos A, Kao CY, Alevriadou BR. Image analysis of dynamic changes in mitochondrial motion and shape inside living vascular endothelial cells: Role of bioenergetics. Presented at the Davis Heart and Lung Research Day, October 2011 (Columbus, OH).

- **Giedt RJ**, Pfeiffer DR, Matzavinos A, Kao CY, Alevriadou BR. Image analysis of dynamic changes in mitochondrial motion and shape inside living vascular endothelial cells: Role of bioenergetics. Presented at the Ohio State University Biomedical Engineering Conference, September 2011 (Columbus, OH).

- **Giedt RJ**, Praetorius-Ibba M, Matzavinos A, Alevriadou BR. Mitochondrial network morphology in post-ischemic vascular endothelial cells. Presented at the Biomedical Engineering Society (BMES) Annual Meeting, October 2010 (Austin, TX).

- **Giedt RJ**, Yang C, Matzavinos A, Praetorius-Ibba M, Zweier JL, Alevriadou BR. Mitochondrial network morphology changes, mechanisms and consequences in postischemic vascular endothelial cells. Presented at the Davis Heart and Lung Institute Research Day, October 2010 (Columbus, OH).

- **Giedt RJ**, Yang C, Matzavinos A, Praetorius-Ibba M, Zweier JL, Alevriadou BR. Mitochondrial network morphology changes, mechanisms and consequences in postischemic vascular endothelial cells. Presented at the Ohio State University Biomedical Engineering Conference, September 2010 (Columbus, OH).

- **Giedt RJ**, Matzavinos A, Alevriadou BR. Analysis of mitochondrial morphology in cells experiencing a heart attack. Presented at the Edward F. Hayes Graduate Research Forum, May 2010 (Columbus, OH).

- **Giedt RJ**, Matzavinos A, Alevriadou BR. Analysis of mitochondrial morphology in postischemic vascular endothelial cells. Presented at the American Heart Association 2010 Young Researchers Reception, April 2010 (Columbus, OH).

- **Giedt RJ**, Jones CI, Alevriadou BR. Mitochondrial superoxide radical generation in endothelial cells exposed to hemodynamic forces. Presented at the Biomedical Engineering Society (BMES) Annual Meeting, October 2009 (Pittsburgh, PA).

- **Giedt RJ**, Jones CI, Galbraith VK, and B.R. Alevriadou. Real-time detection of mitochondrial superoxide radicals in endothelial cells exposed to ischemia/reperfusion injury. Presented at the Ohio State University Biomedical Engineering Conference, May 2009 (Columbus, OH).

- **Giedt RJ,** Jones CI, Galbraith VK, Alevriadou BR. Mitochondrial superoxide levels in endothelial cells exposed to changes in flow and oxygen tension. Presented at The Ohio State University Conference "Engineering and Medicine: The Prescription for an Aging Population", November 2008 (Columbus, OH).

- **Giedt RJ**, Jones CI, Galbraith VK, Alevriadou BR. Mitochondrial superoxide levels in endothelial cells exposed to changes in flow and oxygen tension. Presented at the Davis Heart and Lung Institute Research Day, November 2008 (Columbus OH).

- **Giedt RJ**, Jones CI, Galbraith VK, Alevriadou BR. Mitochondrial superoxide levels in endothelial cells exposed to changes in flow and oxygen tension. Presented at the BMES Annual Meeting, October 2008 (St. Louis, MO).

SERVICE

- 2011 Assistant at University Community Health Care Day – University Hospital East (assisted persons without health care coverage in getting free screenings).

- 2010 Ray Travel Award Judge (Examined graduate student research applications for merit to determine graduate school allocations of travel funds).

- 2007 Brookings County Youth Mentorship Program (Mentored at risk community youth).

PROFESSIONAL MEMBERSHIPS

- 2008-present: Biomedical Engineering Society (BMES).

- 2002-present: American Society of Mechanical Engineers (ASME).

- 2007 Engineering in Training (E.I.T.) Certification.

- 2005 Tau Beta Pi (Engineering Honor Society, awarded to top 1/8 of Junior Class).
- 2004 Pi Tau Sigma (Mechanical Engineering Honor Society, awarded to top 1/4 of Junior Class).